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Detection of quantitative trait loci for locomotion and osteochondrosis-related traits in Large White × Meishan pigs

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Abstract

Data from the F₂ generation of a Large White (LW) × Meishan (MS) crossbred population were analysed to detect quantitative trait loci (QTL) for leg and gait scores, osteochondrosis and physis scores. Legs, feet and gait score were assessed in 308 F₂ animals at 85 (±5) kg and osteochondrosis and physis scores were recorded for the right foreleg after slaughter. A genome scan was performed using 111 genetic markers chosen to span the genome that were genotyped on the F₂ animals and their F₁ parents and purebred grandparents. A QTL on chromosome 1 affecting gait score was significant at the genome-wide significance level. Additional QTL significant at the chromosome-wide 5% threshold level (approx. equivalent to the genome-wide suggestive level) were detected on chromosome 1 for front feet and back legs scores, on chromosome 13 for front legs and front feet scores, on chromosome 14 for front legs, front feet and back legs scores and on chromosome 15 for back feet score. None of the QTL for osteochondrosis score exceeded the chromosome-wide suggestive level, but one chromosome-wide QTL for physis score was found on chromosome 7. On chromosome 1, gait and front feet scores mapped to the middle of the chromosome and showed additive effects in favour of the LW alleles and no dominance effects. The QTL for back legs score mapped to the distal end of the chromosome and showed a dominant effect and no additive effect. On chromosomes 14 and 15, the LW allele was again superior to the MS allele. On chromosome 13, there were both additive and dominance effects in favour of the MS allele. The MS alleles on chromosome 13 may have potential for introgression into a commercial LW population. The other putative QTLs identified may have value in marker-assisted selection in LW or MS-synthetic populations.

Keywords: genetic markers, leg weakness, osteochondrosis, quantitative trait loci, pigs.

Introduction

With the success of genetic improvement of production-related traits, there has been increasing focus in recent years on the improvement of fitness-related traits. There has also been some concern that selective breeding for production traits may have had adverse effects on fitness traits. In pigs, one such fitness trait is leg weakness, which may compromise not only animal welfare but also reproductive performance. Webb *et al.* (1983) and van Sternbergen (1989) cite evidence that between 20 and 50% of

otherwise eligible boars completing a performance test had to be rejected as breeding animals because of leg weakness problems. Lopez-Serrano *et al.* (2000) have demonstrated genetic correlations between leg weakness traits and stayability of breeding sows and suggest that a better leg status would decrease involuntary culling. Several studies such as that of Jørgensen and Andersen (2000) have suggested that osteochondrosis, an inflammatory disease characterized by lesions caused by the necrosis of bone tissue and flaking of the adjacent cartilage, is a

major underlying cause of leg weakness, though Goedegebuure *et al.* (1988) report evidence that osteochondrosis was only a contributory cause of front leg weakness in Duroc pigs. Webb *et al.* (1983), Jørgensen and Andersen (2000), Bereskin (1979) and Rothschild and Christian (1988) have estimated genetic parameters for leg weakness traits and Jørgensen and Andersen (2000) also estimated the genetic relationship between osteochondrosis and leg scores. These authors all conclude that leg score traits have low to moderate heritabilities and might be used in selection indices to reduce leg weakness problems.

With the recent advances in genome mapping, it is now possible to identify chromosomal regions carrying genes influencing quantitative traits. These are known as quantitative trait loci (QTL). The present experiment was designed to investigate QTL for a number of traits in a three-generation cross-breeding programme between two genetically diverse breeds, the European Large White (LW) and the Chinese Meishan (MS). The Meishan and Large White breeds are known to differ significantly for a wide range of traits, including growth rate, fatness, and reproductive performance (Haley and Archibald,

1992). As it is hypothesized that there may be a negative genetic relationship between rapid lean growth and some fitness related traits, and as Large White and Meishan show significant differences in growth and fatness traits, then this cross has the potential to reveal the genes responsible for the putative negative genetic correlations between growth and fitness traits. Quantitative traits were recorded in the F₂ generation. These traits included five leg score traits, observed in the live animals and two bone abnormality scores, osteochondrosis lesions and thickening of the physes (growth plates) in the right forelimb, measured after slaughter and carcass dissection. These seven traits are the focus for the present study.

Material and methods

The animals were taken from a population of approximately 600 F₂ MS × LW pigs, born in three yearly batches. Over the 3 years, a total of 13 grandparent males and 33 grandparent females, with approximately equal numbers of the two purebreeds, and a total 11 F₁ males and 86 F₁ females were used to produce the F₂ population. They were performance tested over a weight range of 30 to

Table 1 (a) Basis of scoring for front and back legs and for front and back feet, on a scale 1 (very poor) to 5 (very good); (b) basis of scoring for gait assessment, on a scale 1 (poor) to 3 (very good); (c) physis score on a scale 0 (normal) to 3 (badly thickened)

Trait	Attribute	Scores				
(a)		5	4	3	2	1
Legs	Strength	v. good	good	moderate	poor	v. poor
	Straightness	v. straight	straight	slightly bent	slight flexing	v. buckled
	Damage	none	none	none	slight -may be temporary	permanent
Feet	Toes-soundness	v. good	good	moderate	poor	v. poor
	Toes-weight distribution	v. even	even	moderate	uneven	v. uneven
	Angle of foot attachment	good	good	moderate	low	v. low
	Damage	none	none	none	slight-may be temporary	permanent
(b)		3	2	1		
Gait	General	v. good	moderate	poor		
	Speed of walk	smooth, quick	moderate	slow		
	Leg movements	smooth, straight	slight splaying or crossing	excessive splaying or crossing		
	Strength of hindquarters	strong	moderate	weak		
	Stability of hindquarters	slight twisting	moderate twisting	excessive twisting		
(c)		0	1	2	3	
Physis		normal thin	slightly thickened	badly thickened	badly thickened with bone damage	

Table 2 Numbers of markers on each chromosome and the position of each marker in centi-Morgans

Chromosome	No. of markers	Marker	Position (cM)	Marker	Position (cM)	Marker	Position (cM)	Marker	Position (cM)	Marker	Position (cM)	Marker	Position (cM)	Marker	Position (cM)	Marker	Position (cM)	Marker	Position (cM)
1	7	SW1515	0.0	CGA	41.9	S0082	69.8	S0155	77.3	SW1828	105.7	SW373	109.1	SW1301	131.2				
2	6	SW2443	0.0	SW256	23.4	SW240	50.6	S0226	74.2	S0378	93.6	S0036	133.0						
3	6	SW72	0.0	SW2527	18.7	SW902	35.9	S0167	67.2	S0002	89.0	SW590	113.3						
4	7	S0227	0.0	S0301	23.3	S0001	42.0	S0217	60.1	S0073	66.9	SW445	97.0	S0097	117.1				
5	8	SW413	0.0	SW491	23.4	SWR453	48.7	DACK	65.1	S0005	79.9	ICFI	103.9	SW1954	117.7	SW967	141.3		
6	7	S0630	0.0	SW2535	9.4	SW1057	50.7	SW782	86.4	S0121	114.9	SW322	146.0	SW2419	158.2				
7	7	S0025	0.0	SW2155	35.1	TNFB	58.3	S0066	79.1	SW632	100.6	S0101	125.8	SW764	148.3				
8	5	SW2611	0.0	S0017	65.2	S0225	81.1	SW61	104.8	S0178	140.8								
9	6	SW983	0.0	SW911	34.5	SW1677	69.8	S0629	89.3	SW2093	96.4	SW1651	156.6						
10	6	SW830	0.0	SW443	31.6	SW497	53.0	SW1041	69.4	SW951	98.3	SWR67	126.8						
11	5	S0385	0.0	SW1632	16.4	S0071	38.3	S0230	47.9	SW703	70.7								
12	4	S0143	0.0	SW957	20.5	S0090	49.2	S0601	86.9										
13	6	SW1378	0.0	S0076	15.0	S0068	52.8	SW398	71.0	SW1056	90.8	S0215	111.7						
14	10	SW857	0.0	SW2496	18.4	SW295	35.8	SW210	44.3	S0007	56.3	SW761	75.5	SW1557	92.9	SW2515	109.0	SWC27	114.2
15	5	S0355	0.0	S0148	14.9	SW964	28.0	SW936	55.0	SW1119	86.8							CYP2E-1	115.9
16	5	S0111	0.0	S0006	48.0	SW403	73.5	S0026	95.7	SW1897	119.4								
17	3	SW24	0.0	SW1920	31.2	S0332	65.1												
18	3	SW2540	0.0	SW1984	29.1	SW1862	41.3												
X	5	SW949	0.0	SW2534	59.2	SW2456	70.4	SW1943	82.4	S0218	93.1								

85 kg. During performance testing, the animals were housed in pens with solid floors and bedded with straw. The first batch and part of the second batch were in pens of four, whilst the rest of batch 2 and the whole of batch 3 were in pens of 13. The three batches were tested at approximately the same season (from January-February to May-July) in each of the 3 years. A commercial pig growth ration was offered *ad libitum* to all three batches. At the end of test, 342 randomly chosen animals, which are the focus of this study, were slaughtered and their carcasses were extensively dissected and many traits including the presence of osteochondrosis lesions and physis score were recorded.

Leg and gait scores

Each animal was assessed at the end of the performance test (85 + 5 kg) for soundness of feet, legs and gait. They were observed as they walked on a concrete pad. The condition of the front legs, front feet, back legs and back feet were each scored on a scale 1 (very poor) to 5 (very good). Soundness of gait was assessed on a scale of 1 (poor) to 3 (very good). All animals were scored by the same person (GBG), who used the attributes listed in Table 1a and b as guidelines in making his assessments. Each 'legs score' is an assessment of the strength of the legs, the straightness and the stability of the joints, whilst each 'feet score' is an assessment of the angle and strength of attachment of the feet to the legs and the soundness of the toes and weight distribution on the toes, both under the pressure of walking. 'Gait score' is an assessment of the speed of walking, together with the ease and smoothness of leg movement and the steadiness of the body, especially the hindquarters whilst walking.

Osteochondrosis lesions and physis scores in the right forelimb

As part of the carcass dissection procedure, the right forelimb from each carcass was examined after the removal of skin and muscle. The articular surfaces of the scapula, proximal and distal humerus and proximal radius and ulna were inspected along with distal physes (growth plates) of the radius and ulna and the presence of osteochondrosis (OCD) lesions were noted. Photographs were taken of the distal ulnar-radial physes and used to score their appearance. This was scored as indicated in Table 1c.

Genotyping

A total of 308 F₂ individuals (178 entire males and 130 females) and their F₁ parents and purebred LW and MS grandparents, were genotyped by a

commercial genotyping company. The markers were selected in advance and the criteria for selection were: (1) to provide cover of the whole genome at 20 to 25 centiMorgans (cM) between markers; (2) to be informative in the population; (3) to be technically tractable with no known null alleles; and (4) to have been used in other studies. The marker maps were developed using Cri-map (Green *et al.*, 1990). The markers chosen and their estimated map positions are shown in Table 2. Details of the microsatellite markers used, PCR primer sequences and PCR conditions can be found in the ARKdb-pig genome database (<http://www.thearkdb.org/pig>). The marker genotypes and trait data were entered into the project database (<http://www.resSpecies.org>).

Least squares analyses

Analyses of trait data and estimation of correlations between traits were performed using the GenStat statistical package (GenStat, 2000).

QTL analyses

All analyses were performed using the methods devised by Haley *et al.* (1994) as implemented in the software QTL Express (Seaton *et al.*, 2002). The assumption is made in these analyses that the detectable QTL were fixed for alternative alleles in the MS and LW grandparental breeds. Some of the traits analysed are categorical, rather than being continuously distributed. However, previous studies have shown that the methods of analysis used can be used even for data in just two classes with little loss of power and no substantially increased risk of type I errors or biases in the estimated positions of QTL (Visscher *et al.*, 1996).

The basic model for the analysis of leg and gait scores fitted sex and date of measurement as classified factors and age in days at measurement (end of test) as a covariate. Date of measurement had a total of 20 levels over the 3 years. Since animals were scored at a fixed weight, the age effect is also an estimate of the effect of growth rate so this latter effect was not included as a separate covariate. The model for osteochondrosis lesions and physis score fitted sex, yearly batch (three levels) and age. Analyses fitting extra co-variables, P2 fat thickness (subcutaneous fat taken at 6 cm from the mid-dorsal line at the last rib), leg length and body length were additionally performed where each of these traits was shown in covariance analyses to have a significant effect on the trait being analysed.

For each trait, the regression model was fitted at 1-cM intervals along each chromosome and the F value

for the QTL effect calculated at each point. The results of this procedure are illustrated for three traits on one chromosome in Figure 1. The position of the QTL effect was taken to be that with the highest F value. At this point, an additive effect (a), which is an estimate of the effect of one copy of a LW allele compared with a MS allele, and dominance effect (d), which was the deviation of a heterozygote from the homozygote mean, were estimated. In the results, a positive additive effect indicates that the LW allele increases the trait values and a negative effect indicates that the MS allele increases the trait values. A dominance effect with the same sign as the additive effect indicates that the LW allele is dominant, and with the opposite sign that the MS allele is dominant. In this context it should be remembered that OCD and physis score are recorded on a descending scale and thus a low score is desirable.

Where evidence was found for a single QTL on a chromosome, further analyses were performed. Firstly we fitted a model with two linked QTL on that chromosome. The best two-linked QTL model was identified by a grid search at 1-cM resolution of all possible positions for two QTL, the two positions that maximized the joint F value testing the model of two QTL *versus* no QTL being chosen. The significance of the second QTL is judged by deriving the F value for the comparison of the best two-QTL model *versus* the best single-QTL model for that linkage group. This F value is tested against significance thresholds derived for the tests of one QTL *versus* no QTL, as has previously been found to be appropriate (de Koning, 2001). Secondly we looked for evidence that the effect of a single QTL differed between the two sexes by fitting an interaction between the QTL effect and sex (see for example Knott *et al.*, 1998).

The proportion of the phenotypic variance explained by individual QTL was estimated from the reduction in the residual mean square resulting from the inclusion of the QTL effect in the analysis.

Three significance threshold levels were set. The genome-wide level is equivalent to a 5% type I rate in the entire genome, whilst the chromosome-wide threshold is the similar level over a single chromosome. As there are 19 independent chromosomes scanned, a 5% chromosomal threshold leads to an expectation of observing 0.95 QTL significant at this level in a scan covering all chromosomes, which is approximately equivalent to the genome-wide suggestive level of significance. Finally there is a nominal level, set such that the 5%

Table 3 The observed distribution of legs, feet and gait scores, (legs and feet on a scale 1 (poor) to 5 (good) and gait on a scale 1 (poor) to 3 (good)) in the live animal and osteochondrosis lesions (absence or presence) and physis score (scale 0 (normal) to 3 (badly thickened)) following carcass dissection

	Good				Poor
	5	4	3	2	1
Front legs score	0	54	230	21	3
Front feet score	0	81	197	30	0
Back legs score	0	34	246	25	3
Back feet score	0	48	241	15	4
	Good		Poor		
	3	2	1		
Gait	26	229	53		
	Absence		Presence		
	0	1			
Osteochondrosis lesions	252	56			
	Normal		Badly thickened		
	0	1	2	3	
Physis score	64	113	62	10	

type I error rate is expected at a single point on a chromosome.

Results

Overall distributions of scores

There was a total of 308 F₂ animals with genotype and legs/gait score records. The overall distribution of scores is shown in Table 3. For each trait, the intermediate score was by far the largest category and no animals were given the score of 5 for legs or feet.

As also shown in Table 3, osteochondrosis (OCD) lesions were observed in about one-fifth of right forelimbs dissected and the trait was analysed as absence (0) or presence (1). The photographs of the distal ulnar/radial physis were not interpretable for 59 animals leaving 249 physis score records for analysis. The distribution, shown in Table 3, indicates that the majority of animals had some problems.

Overall correlations

Correlations were estimated among the above traits and with some potentially related traits. These were age at measurement of leg score (end of performance test), growth rate from weaning to the end of test, hind leg length, body length and P2 fat depth, the last three being recorded in the carcass dissection procedure. For this sample size, the values needed

for a correlation to be greater than zero are 0.11 ($P < 0.05$) and 0.16 ($P < 0.01$).

Correlations between OCD lesions and all the other traits were close to zero, the greatest being -0.13 with P2 fat depth. Physis score had small negative correlations with front and back leg scores and with P2 fat depth (range -0.15 to -0.11), suggesting that the physis was more severely damaged in animals with lower (poorer) leg scores and in leaner animals. Physis damage tended to be greater in tall and long animals, the correlations with leg length being $+0.20$ and with body length $+0.13$.

All correlations among the five feet, leg and gait scores were positive (range $+0.08$ to $+0.46$). All five scores were negatively correlated with growth rate, especially front feet (-0.25) and gait (-0.20), suggesting that faster growth led to more leg problems. In line with the results for the physis score, taller and longer animals suffered more feet, leg and gait problems, the largest correlations being between back leg score and leg length (-0.15), gait score and leg length (-0.14) and gait score and body length (-0.17). Fatter animals tended to have lower feet scores, P2 fat depth being negatively correlated with the feet scores (front -0.14 , back -0.13).

Linear models

The effects of potential co-variables were examined by least-squares analysis. The dependent variates were the OCD lesions, physis, leg, feet and gait scores, with the other traits being fitted as co-variables in various combinations. The sex and the yearly batch were included as classified factors in all the models. The results are summarized below highlighting those effects which were significant ($P < 0.05$) or approached significance ($P < 0.10$). OCD lesions were present in fewer females than males ($P < 0.001$) and females had lower physis scores ($P = 0.007$), but sex had no effect on any of the leg or gait scores. Batch (year) effects had highly significant effects ($P < 0.01$) on four of the seven traits, but there was no consistent pattern across batches. Batch effects tended to decline in models where more co-variables, such as growth rate, were added. Animals with high growth rates tended to be more prone to OCD lesions ($P = 0.065$) and had poorer front legs ($P = 0.097$) and front feet ($P = 0.007$), than those growing more slowly. Increased leg length was associated with higher physis scores ($P = 0.012$) and lower back leg and gait scores ($P = 0.008$ for both traits). Body length had no significant associations with any of the dependant variables. There was a negative relationship between fatness level, as

depicted by the P2 measurement and front feet ($P = 0.038$) and back feet ($P = 0.090$) scores.

Markers and linkage analyses

A total of 111 markers, largely microsatellites, were used. Maps were developed from published consensus maps using Cri-Map (Green et al., 1990) with marker order confirmed against alternative permutations using the flips option. The resulting sex-averaged map distances are shown in Table 2. These maps do not differ substantially from other published consensus maps (e.g. see ARKdb-pig genome database (<http://www.thearkdb.org/pig>)) and span a total of 21.57 Morgans with an average distance of 23 cM between markers.

QTL analyses

The significance levels for the chromosomal and genome-wide threshold levels were determined by permutation (Churchill and Doerge, 1994) using 5000 permutations. We found little variation in the threshold across different traits (and relatively little across different chromosomes for the chromosomal threshold) and so chose to use the same value for all traits and chromosomes. These were; nominal 5%, 3.0, chromosomal 5%, 5.0, genome 5%, 9.0 and genome 1%, 11.0 for an analysis without sex interactions.

The quoted results for the QTL analyses were those estimated using the models described earlier. Analyses fitting extra covariates as indicated by the linear models analyses in the previous section were performed, e.g. leg length was added in analyses of back leg, gait and physis scores. In general, fitting these extra covariates tended to reduce the F-ratios slightly but had little impact on the size and direction of the QTL estimates.

At the nominal 5% level, QTL effects for at least one of the seven traits were observed on all the chromosomes except 11, 12, 17 and X. Most estimated QTL effects appeared to be isolated effects on one individual trait showing no relation to other traits. There were however some chromosomal regions, which influenced more than one of the traits, notably on chromosome 1, but also on chromosomes 13, 14 and 15. The results, which reached the nominal significance level, are shown for these four chromosomes in Table 4, listed by chromosome, and those for the other chromosomes are given in Table 5 listed by trait. For brevity in the following text the location of the peak F value for each QTL is given as the relevant position expressed in centiMorgans (cM) on the linkage maps developed in the current population. These positions are specific to this cross. The information required to transfer these QTL

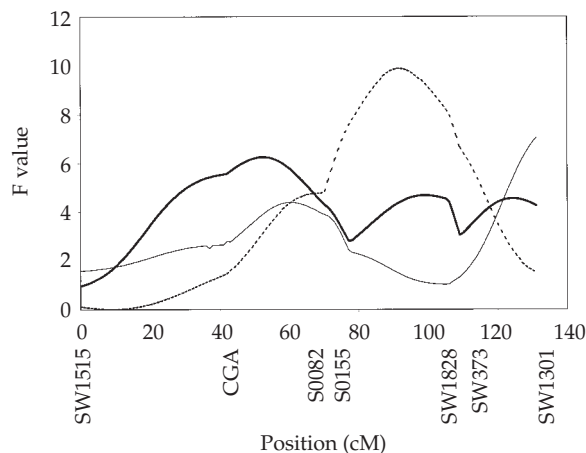


Figure 1 F values from the QTL analyses for front feet (—), back legs (---) and gait scores (.....) for all positions on chromosome 1.

results to other populations are the marker intervals. The markers flanking each QTL peak are listed in Tables 4 and 5 and their overall position can be ascertained from Table 2.

Chromosome 1

These results are shown in Table 4 and those for three traits are illustrated in Figure 1. Single-QTL analyses showed nominal significant effects for all traits except OCD lesions and a second significant QTL was detected for two traits. The largest single-QTL effect and the only one to reach the genome-wide threshold was for gait score at 92 cM, with an additive effect in favour of the LW allele and no significant dominance effect. Single-QTL analyses also suggested a nominally significant effect at 94 cM for front leg score. Significant effects at the chromosomal level in favour of the LW allele were revealed for front feet (52 cM) and at the nominal level for back feet (72 cM). A single QTL for back legs (131 cM) was significant at the chromosomal threshold, having no significant additive effect and a significant dominance component in the direction of improving the leg score of the heterozygote compared to the mid-parent value. Two-QTL analyses for front legs, front feet, back legs and back feet all suggested the best positions to be in the 50-70 cM region and at 131 cM. However the second QTL was only significant at the nominal level for back legs and back feet. At 50-70 cM, there was an additive effect in favour of the LW allele, whilst at 131 cM, there was no significant additive effect, but a significant dominance effect for both back legs and back feet in the direction of improving the scores of the heterozygote compared with the mid-parent

value. For physis score, a single-QTL effect was significant at the nominal level (100 cM), having no significant additive effect, but a significant dominance effect, reducing (improving) physis score in the heterozygote, compared with the mid-parent value.

Chromosome 13

The results for chromosome 13 are shown in Table 4. Single-QTL analyses revealed significant effects at the chromosomal level for front legs (58 cM) and front feet (56 cM) and at the nominal level for physis score (53 cM). For front feet, there was an additive effect in favour of the MS allele. For the three traits the MS allele showed dominance effects, expressed principally in the male; increasing front leg and feet scores and lowering physis score in the heterozygote compared to the mid-parent value. A little further along the chromosome, there was a nominally significant additive effect for gait score (70 cM), again in favour of the MS allele. A two-QTL analysis of gait score revealed a further effect, again only at the

nominal level, at the top end of the chromosome (0 cM) in addition to the one at 70 cM. The QTL at 0 cM had a significant additive effect in favour of the LW allele.

Chromosome 14

The results for chromosome 14 are shown in Table 4. Near the top end of the chromosome, there were significant effects at the chromosomal level for front legs (21 cM) and front feet (4 cM) and at the nominal level for back feet (8 cM). In all three cases, there was an additive effect in favour of the LW allele, that for front feet being expressed more strongly in the female. Further down the chromosome, there was a significant effect at the chromosomal level for back legs (38 cM), with significant additive and dominance effects for the LW allele. There was also evidence of a QTL for gait score further down the chromosome (109 cM), with a dominance effect which improved the heterozygote in comparison with the mid-parent value.

Table 4 Significant (exceeding the nominal 5% threshold) QTL effects on chromosomes 1, 13, 14 and 15

No. of QTL in model	Trait	Position (cM)	F value	Prop. var. explained	Additive † effect	Dominance † effect	Flanking markers
Chromosome 1							
1	Front legs	94	4.49	0.026	0.166**	0.075	S0155 – SW1828
1	Front feet	52	6.25	0.035	0.204***	-0.006	CGA – S0082
1	Back legs	131	7.05	0.041	0.042	0.226 ***	SW1301
2	Back legs	63/131	4.08‡	0.061	0.121	-0.100	CGA – S0082
					0.027	0.224 ***	SW1301
1	Back feet	72	4.39	0.023	0.125**	-0.076	S0082 – S0155
2	Back feet	72/131	3.63‡	0.041	0.124**	-0.030	S0082 – S0155
					0.042	0.165 *	SW1301
1	Gait	92	9.88	0.058	0.231***	0.014	S0155 – SW1828
1	Physis score	100	3.64	0.021	-0.067	-0.394 **	S0155 – SW1828
Chromosome 13							
1	Front legs	58	5.39	0.030	-0.060	0.230 **	S0068 – SW398
1	Front feet	56	6.51	0.037	-0.143**	0.170 *	S0068 – SW398
1	Gait	72	3.33	0.016	-0.120*	-0.016	SW398 – SW1056
2	Gait	0/70	3.31‡	0.030	0.105*	-0.062	SW1378
					-0.126**	-0.026	S0068 – SW398
1	Physis score	53	3.08	0.017	0.094	-0.256 *	S0068 – SW398
Chromosome 14							
1	Front legs	21	5.54	0.031	0.101*	0.170 *	SW2496 – SW295
1	Front feet	4	6.76	0.039	0.181***	-0.079	SW857 – SW2496
1	Back legs	38	5.99	0.034	0.109*	0.132 *	SW295 – SW210
1	Back feet	8	3.53	0.017	0.127**	-0.017	SW857 – SW2496
1	Gait	109	3.13	0.014	0.037	0.134 *	SW2515
Chromosome 15							
1	Back legs	14	4.36	0.023	0.115*	0.089	S0355 – S0148
1	Back feet	8	5.06	0.028	0.155**	-0.073	S0355 – S0148

† For two-QTL models, additive and dominance effects and flanking markers are shown for each QTL.

‡ For two-QTL models, the significance of the second QTL effect is shown.

Table 5 Significant (exceeding the nominal 5% threshold) QTL effects on chromosomes other than 1, 13, 14 and 15, listed by trait

Trait	Chromosome	Position (cM)	F value	Prop. var. explained	Additive effect	Dominance effect	Flanking markers
Front legs	18	28	3.21	0.015	0.120 *	-0.037	SW2540 – SW1984
Front feet	5	61	3.63	0.018	-0.068	0.190 *	SWR453 – DAGK
	9	0	3.97	0.021	-0.089	-0.234 *	SW983
Back legs	2	78	4.61	0.025	0.091	0.180 *	S0226 – S0378
	3	73	6.12	0.035	0.090	0.190 **	S0167 – S0002
	7	66	3.00	0.014	0.092	-0.138	TNFB – S0066
	10	126	3.70	0.019	0.116 *	0.041	SWR67
	16	10	4.05	0.021	-0.037	0.460 **	S0111 – S0006
Gait	2	6	6.95	0.040	-0.113 *	-0.226 **	SW2443 – SW256
	4	23	7.35	0.042	0.100 *	0.210 **	S0301
	5	141	5.41	0.030	-0.035	-0.208 **	SW967
	6	154	3.25	0.016	0.006	-0.191 **	SW322 – SW2419
	8	0	3.83	0.019	-0.041	-0.173 *	SW2611
	10	62	6.57	0.038	0.128 *	-0.234 **	SW497 – SW1041
OCD lesions	7	132	3.07	0.013	-0.003	0.155 *	S0101 – SW764
	16	52	3.29	0.015	0.006	-0.171 *	S0006 – SW403
Physis score	3	53	4.55	0.028	-0.160	0.460 *	SW902 – S0167
	4	55	4.65	0.029	-0.150 *	0.340 *	S0001 – S0217
	6	73	3.18	0.018	-0.005	0.534 *	SW1057 – SW782
	7	68	8.68	0.059	-0.380 ***	0.211	TNFB – S0066
	8	89	4.22	0.026	-0.009	-0.438 **	S0225 – SW61
	16	110	3.89	0.023	0.074	0.288 **	S0026 – SW1897

Chromosome 15

Near the top end, there were significant effects observed for back legs (14 cM) and back feet (8 cM), both near to the chromosomal threshold. In both cases, the effects were additive in favour of the LW allele.

Other chromosomes

A list of the effects detected at the nominal level on other chromosomes is given in Table 5. It can be seen that most of the F-ratios were below the chromosomal threshold and that the positions of the effects bear little relationship to each other. In addition, comparatively few showed significant additive effects. The analyses detected one QTL for back legs within the chromosomal threshold, on chromosome 3 (73 cM), where a dominance effect was observed. Four gait score chromosomal level QTL were found, on chromosome 2 (6 cM), where there was a significant additive effect for the MS allele, which also showed a dominant effect, on chromosome 4 (23 cM) where the LW allele had both positive additive and dominance effects, on chromosome 5 (141 cM) where there was a dominance effect and on chromosome 10 (62 cM) where the LW allele had a positive additive effect, but the MS allele showed dominance. One QTL for physis score was detected at the chromosomal level, on chromosome 7 (68 cM), with an additive effect in favour of the LW allele.

Discussion

This paper reports only the second QTL study in pigs of traits associated with leg weakness. As mentioned in the introduction, it may be possible to relate QTL for leg weakness traits to those for growth and carcass traits. The data for such traits in the current population has yet to be analysed fully and will form part of a future publication, which may include bivariate analyses of growth with leg weakness and other fitness traits. The present discussion will therefore attempt to link the position of the observed leg weakness QTL in the current population with those for growth and fatness traits reported in the literature. A QTL significant at the genome-wide level affecting gait was identified on chromosome 1 and QTL significant at the suggestive level were detected on several other chromosomes with clusters of effects on chromosomes 1, 13, 14 and 15. The observed leg scores for individual animals indicate the presence of some locomotion deficiency for all the animals in this Meishan X Large White F₂ population, since none was given a very good (5) score for any of the leg and feet scores. High incidences of leg and feet troubles were also reported by Jørgensen and Andersen (2000) and reflect the potential severity of the problem. The results section focussed on those chromosomal regions that are associated with significant effects on more than one trait. As discussed earlier, previous studies suggest

that conditions, such as osteochondrosis, are important underlying causes of leg weakness traits. If a QTL was acting through malfunctions of bone or joint action then this might be expressed in more than one of the recorded scores. Thus, it was disappointing that none of the osteochondrosis effects reached the chromosomal significance level, but this may be due to a relatively low occurrence (18% of the total) and the moderate number of animals studied. The frequency of osteochondrosis effects is similar to the levels in the humeral condyles in Yorkshire pigs reported by Jørgensen and Andersen (2000) but these authors found that occurrence of OCD lesions in the front legs and particularly in the humeral condyles was much lower than in sites in the back leg, especially in the femoral condyles. Andersson-Eklund *et al.* (2000) also observed fewer incidences of osteochondrosis in the humerus than in the femur in a wild boar \times Large White population. These authors found QTL for osteochondrosis on chromosomes 5, 13 and 15, with the wild boar alleles reducing the level of osteochondrosis. Although these are at different positions to those for osteochondrosis in the present study, their QTL on chromosome 13 was in a similar region to the observed QTL for front legs, front feet, gait and physis scores (53 to 72 cM, flanked by markers S0068 and SW398) in the present study, suggesting a possible underlying connection between osteochondrosis and leg scores. Another similarity was that the LW allele had an inferior effect to the MS as it did to the wild boar in the Swedish study. In this region of chromosome 13, Andersson *et al.* (1994) had earlier identified a QTL affecting early growth rate in the same population. The POU2F1 gene, which has a regulatory effect on growth hormone production and has been shown by Yu *et al.* (1999) to influence early growth rate traits, maps to this region between markers S0068 and SW398. Bidanal *et al.* (2001) also detected a QTL for early growth rate in a similar region of chromosome 13. The action of such growth genes might be expected to have an effect, directly or indirectly, on leg score traits, especially as fast growing animals in the present trial had poorer front leg and feet scores and were more prone to osteochondrosis than those growing more slowly.

The results for chromosome 1 suggest the presence of two QTL regions. The first was at 52 to 94 cM, a region flanked by marker CGA through to S0082, S0155 and SW1828. In this region, gait score showed significant effects at the genome level, front feet at the chromosomal level and front legs and back feet at the nominal level. In each case, there was an additive effect in favour of the LW allele with no significant dominance effect. There may therefore be a QTL in

this region affecting bone or joint structure and thus mobility generally. At the end of the chromosome, near marker SW1301, there was evidence for a second QTL. Here there was a significant effect at the chromosomal level for back legs and the two QTL analyses for all four feet and leg traits suggested a QTL at this point. For each trait the additive effect was not significant, but there was significant dominance. The nominally significant QTL for physis score at 100 cM behaved in a way which suggested that it may be linked to the second QTL region in that there was no significant additive effect but there was a dominance effect, reducing (improving) physis score. In this region of chromosome 1 (between SW373 and SW1301), Paszek *et al.* (1999) and Bidanal *et al.* (2001) both reported a QTL for daily gain, where both additive and dominance effects were observed. De Koning *et al.* (1999) found a QTL for backfat thickness near the end of chromosome 1. This showed no significant additive effect, but there was a large dominance effect, a pattern similar to that of the QTL in the present population. Bidanal *et al.* (2001) also found a fatness QTL in this region where the MS allele increased fatness, but the LW allele was dominant. There could be a possible link between the action of these genes and the QTL detected here.

The results for chromosome 14 suggest a possible QTL region flanked by the first and fourth markers, SW857 and SW210. There may be two genes acting here, since the effects for leg scores peaked at 21 to 38 cM, between SW2496, SW295 and SW210 and had small additive and dominance effects in favour of the LW, whereas those for feet scores are at 4 to 8 cM, between SW857 and SW2496, and show stronger additive effects but no dominance effects. There may be separate effects for leg action and feet soundness. The observed QTL on chromosome 15 is between the first two markers, S0355 and S0148, and had additive effects for back legs and back feet score both in favour of the LW allele. It therefore may have associations with hip or back leg action.

Of the effects on other chromosomes, listed in Table 5, the two with the highest significance levels were physis score at 68 cM on chromosome 7 (flanked by TNFB and S0066) and gait score at 23 cM on chromosome 4 (close to S0301). Both these chromosomes have been extensively researched for QTL affecting growth, fatness and body dimensions. The observed QTL for physis score on chromosome 7 is in a region where Rohrer (2000) amongst others has detected a QTL, where the MS allele is associated with faster growth rate, reduced fatness and greater body length. It is possible that such a gene may directly or indirectly lead to greater physis damage

associated with the MS allele in the current results. The observed QTL for gait score on chromosome 4 is outside the region where growth rate QTLs have been reported, for example by Walling *et al.* (2000), so no inferences can be made in this case.

In this experiment the QTL regions detected were on the basis of the differences between alleles originating in Meishan and Large White pigs. Apart from chromosome 13, Large White alleles were observed to have the most prominent additive effects in the highlighted regions. Since the Large White is the indigenous commercial population, the chromosome 13 QTL would offer the only scope for improving feet and leg traits in this population by the incorporation of Meishan genes by marker assisted introgression. The study does give an indication of chromosomal regions where genes for locomotion traits exist. Further work would be needed to refine the position of the markers, especially on chromosome 1, but it may be possible to identify genes segregating in Large White or Large White \times Meishan synthetic populations in these regions and incorporate these in marker assisted selection programs. As previously mentioned, leg weakness in pigs and indeed in other livestock species, has featured in many husbandry and quantitative genetic studies and a molecular analysis provides an additional milestone in the understanding of the problem and its underlying causes.

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References

- Andersson, L., Haley, C. S., Ellegren, H., Knott, S. A., Johansson, M., Andersson, K., Andersson-Eklund, L., Edforslilja, I., Fredholm, M., Hansson, I., Hakansson, J. and Lundstrom, K. 1994. Genetic mapping of quantitative trait loci for growth and fatness in pigs. *Science* **263**: 1771-1774.
- Andersson-Eklund, L., Uhlhorn, H., Lundeheim, N., Dalin, G. and Andersson, L. 2000. Mapping quantitative trait loci for principal components of bone measurements and osteochondrosis scores in a wild boar \times Large White intercross. *Genetical Research* **75**: 223-230.
- Bereskin, B. 1979. Genetic aspects of feet and leg soundness in swine. *Journal of Animal Science* **48**: 1322-1328.
- Bidanal, J. -P., Milan, D., Iannuccelli, N., Amigues, Y., Boscher, M. -Y., Bourgeois, F., Caritez, J. -C., Gruand, J., Le Roy, P., Lagant, H., Quintanilla, R., Renard, C., Gellin, J., Ollivier, L. and Chevalet, C. 2001. Detection of quantitative trait loci for growth and fatness in pigs. *Genetics, Selection, Evolution* **33**: 289-309.
- Churchill, G. A. and Doerge, R. W. 1994. Empirical threshold values for quantitative trait mapping. *Genetics* **138**: 963-971.
- GenStat. 2000. *GenStat for Windows, release 4.2, fifth edition*. VSN International Ltd, Oxford.
- Goedegebuure, S. A., Rothschild, M. F., Christian, L. L. and Ross, R. F. 1988. Severity of osteochondrosis in three genetic lines of pigs selected for front leg weakness. *Livestock Production Science* **19**: 487-498.
- Green, P., Falls, K. and Crooks, S. 1990. *Crimap version 2.4*. Washington University School of Medicine, St Louis.
- Haley, C. S. and Archibald, A. L. 1992. Porcine genome analysis. In *Genome analysis* (ed. K. E. Davies and S. M. Tilghman), pp. 99-129. Cold Spring Harbor Laboratory Press, New York.
- Haley, C. S., Knott, S. A. and Elsen, J. M. 1994. Mapping QTL in crosses between outbred lines using least squares. *Genetics* **136**: 1195-1207.
- Jorgensen, B. and Andersen, S. 2000. Genetic parameters for osteochondrosis in Danish Landrace and Yorkshire boars and correlations with leg weakness and production traits. *Animal Science* **71**: 427-434.
- Knott, S. A., Marklund, L., Haley, C. S., Andersson, K., Davies, W., Ellegren, H., Fredholm, M., Hoyheim, B., Hansson, I., Lundstrom, K., Moller, M. and Andersson, L. 1998. Multiple marker mapping of quantitative trait loci in an outbred cross between wild boar and Large White pigs. *Genetics* **149**: 1069-1080.
- Koning, D. -J. de. 2001. Identification of (non-) Mendelian factors affecting pork production. *Ph. D. thesis, Wageningen University*.
- Koning, D.-J. de, Janss, L. L. G., Rattink, A. P., Oers, P. A. M. van, Vries, B. J. de, Groenen, M. A. M., Poel, J. J. van der, Groet, P. N. de, Brascamp, E. W. and Arendonk, J. A. M. van. 1999. Detection of quantitative trait loci for backfat thickness and intramuscular fat content in pigs. *Genetics* **152**: 1679-1690.
- Lopez-Serrano, M., Reinsch, N., Looft, H. and Kalm, E. 2000. Genetic correlations of growth, backfat thickness and exterior with stayability in Large White and Landrace sows. *Livestock Production Science* **64**: 121-131.
- Paszek, A. A., Wilkie, P. J., Flickinger, G. H., Rohrer, G. A., Alexander, L. J., Beattie, C. W. and Schook, L. B. 1999. Interval mapping of growth in a divergent swine cross. *Mammalian Genome* **10**: 117-122.
- Rohrer, G. A. 2000. Identification of quantitative trait loci affecting birth characters and accumulation of back fat and weight in a Meishan-White Composite resource population. *Journal of Animal Science* **78**: 2547-2553.
- Rothschild, M. F. and Christian, L. L. 1988. Genetic control of front-leg weakness in Duroc swine. 1. Direct response to five generations of selection. *Livestock Production Science* **19**: 459-471.

- Seaton, G., Haley, C. S., Knott, S. A., Kearsley, M. and Visscher, P. M. 2002. QTL Express: mapping quantitative trait loci in simple and complex pedigrees. *Bioinformatics* **18**: 339-340.
- Sternberger, E. J. van. 1989. Description and evaluation of a linear scoring system for exterior traits in pigs. *Livestock Production Science* **23**:163-181.
- Visscher, P. M., Haley, C. S. and Knott, S. A. 1996. Mapping QTLs for binary traits in backcross and F₂ populations. *Genetical Research* **68**: 55-63.
- Walling, G. A., Vischer, P. M., Andersson, L., Rothschild, M. F., Wang, L., Moser, G., Groenen, M. A. M., Bidanal, J. - P., Cepica, S., Archibald, A. L., Geldermann, H., Koning, D. -J., Milan, D. and Haley, C. S. 2000. Combined analysis of data from quantitative trait loci mapping studies: chromosome 4 effects on porcine growth and fatness. *Genetics* **155**: 1369-1378.
- Webb, A. J., Russell, W. S. and Sales, D. I. 1983. Genetics of leg weakness in performance-tested boars. *Animal Production* **36**: 117-130.
- Yu, T. P., Wang, L., Tuggle, C. K. and Rothschild, M. F. 1999. Mapping genes for fatness and growth on pig chromosome 13: a search in the region close to the pig PIT1 gene. *Journal of Animal Breeding and Genetics* **116**: 269-280.

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